REMARKS

Claims 1-37 are pending. Claim 38 has been withdrawn from consideration. Favorable consideration and allowance are respectfully requested for claims 1-37 in view of the following remarks.

Claim Rejections – 35 U.S.C. §103(a)

Claims 1-4, 14-31, 33 and 35-37 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Gitan *et al.* (*Genome Research*, 2001, 12, 158-164) in view of Bransteitter *et al.* (*PNAS*, 2003, 100, 4102-4107).

According to the Examiner, Gitan *et al.* purportedly teach a method for detecting the presence or level of alkylated cytosine in a sample of genomic double stranded DNA from an individual, the method comprising the following steps:

(a) obtaining a sample of the genomic double stranded DNA from the individual;

(b) converting at least one region of the double stranded DNA to single stranded DNA by treating with mild heat and alkali/sodium bisulfite using the Integren CpGenome DNA modification kit;

(c) differentially modifying alkylated cytosine and cytosine present in single stranded DNA (Gitan *et* al. teach treating with bisulfite converts unmethylated cytosine to uracil, wherein methylated cytosine remains unchanged); and

(d) determining the level of bisulfite modification of the target region by analyzing converted and unconverted alleles on an oligonucleotide array.

The Examiner acknowledges that Gitan *et al.* are silent in relation to the use of an enzyme to differentially modify alkylated cytosine and cytosine present in single stranded DNA. However, the Examiner alleges that an enzyme capable of differentially modifying alkylated cytosine and cytosine was known in the art at the time of the claimed invention (see Bransteitter *et al.*). According to the Examiner, Bransteitter *et al.* teach a method wherein an Activation Induced Cytidine Deaminase (AID) enzyme differentially modulates that activity of single stranded DNA comprising cytosine and methylated cytosine.

Therefore, the Examiner has formed the view that it would be allegedly obvious to one skilled in the art at the time the present invention was made to combine the teachings of Gitan *et al.* and Bransteitter *et al.* to arrive at a method for detecting the presence of alkylated cytosine in a sample of genomic or mitochondrial double stranded DNA. Applicants respectfully disagree.

To establish a *prima facie* case of obviousness, three basic criteria must first be met. First, there must be some suggestion of modification, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all of the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on the Applicant's disclosure. *In re Vaeck,* 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) (emphasis added).

Gitan *et al.* describe an assay in which the bisulfite treatment of genomic DNA deaminates unmethylated cytosine bases in the nucleic acid template to uracil, whereas 5-methylcytosine is resistant to deamination. The bisulfite incubation protocol used by Gitan *et al.* uses incubation at high molar NaOH to denature double stranded DNA to single stranded DNA (see ***attached*** as Appendix I the CpGenome DNA modification kit instruction pamphlet, in particular page 7 of the instruction pamphlet, Step 2, which describes the DNA modification procedure). Here is it described that the DNA is added to 3M NaOH to a final concentration of approximately 0.2M NaOH. Such a concentration results in a mixture having a pH of approximately 13.3. This mixture is then incubated for prolonged periods (i.e. up to 16 hours) at 50°C.

The Examiner alleges that it would have been *prima facie* obvious for one of skill in the art to substitute the bisulfite step in Gitan *et al.* with the Activation-Induced Deaminase (AID) enzyme in Bransteitter *et al.* with a reasonable expectation of success. Applicants respectfully disagree.

Section 2143.01 of the Manual of patent Examining Procedures (M.P.E.P.) states:

*"**The proposed modification cannot render the prior art unsatisfactory for its intended purpose***

If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. In re Gordon, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984)" (emphasis added)

It is respectfully submitted that modification of the method of Gitan *et al.* to substitute the bisulfite reaction with an enzyme, such as AID, would render the prior art as unsatisfactory for its intended use. For example, Step 2 of the DNA Modification procedure used by Gitan *et al.* describes mixing 3M NaOH and DNA in water, wherein the resultant mixture has a final NaOH concentration of approximately 0.2M and a pH of approximately 13.3. The resultant mixture is then incubated for 16 hours at 50°C.

Temperature and pH are environmental factors important to the activity of an enzyme. Enzymes have a protein and non-protein (co-factor) portion. The bond which holds the enzyme in its structure is destroyed by changes in pH and temperature. The optimal pH value for most enzymes falls in the range of pH 6-8, although there are some enzymes which are capable of working at more acidic environments, such as those of the digestive tract. However, enzymatic function is lost in strongly basic environments. Similar issues also arise when maintaining enzymes at elevated temperatures, such as those temperatures taught in the methods of Gitan *et al.* As such, it is respectfully submitted that the use of an enzyme in the methods taught by Gitan *et al.* would result in the modification of the prior art such that it was unsatisfactory for its intended purpose i.e. the enzyme would be degraded and the level of methylation not ascertainable.

Therefore, in order for the method of Gitan *et al.* to work whilst using an enzyme to detect methylation, the method would require substantial reconstruction. According to M.P.E.P section 2143.01:

"If the proposed modification or combination of the prior art would change the principle of operation of the prior art being modified, then the teachings of the reference are not sufficient to render the claims prima facie obvious. In re ratti, 270 F.2d 810, 123 USPQ 349 (CCPA 1959)...the "suggested combination of references would require a substantial reconstruction and redesign of the elements shown in [the primary reference] as well as a change in the basic principle under which the [primary reference] construction was designed to operate."

Here, the Court held that the substantial reconstruction and redesign of the prior art meant that the teachings of the prior art were not sufficient to render the claims are *prima facie* obvious.

It is submitted that in order for the methods of Gitan *et al.* to work with an enzyme such as AID, the methods would require a substantial reconstruction and re-design. As such, it respectfully submitted for at least these reasons that the combination of Gitan *et al.* and Bransteitter *et al.* does not render the present invention as obvious.

Further, as described in the Background section of the present specification there are inherent issues with bisulfite reactions. Artefacts arise as not all cytosines are converted to uracil, small percentages of 5-methylcytosine are converted to thymidine and DNA is lost due to fragmentation. These are caused by long incubation periods and the use of non-physiological conditions, such as basic pH. Accordingly, in light of the limitations of bisulfite methods, it is respectfully submitted that the skilled artisan would not combine Gitan *et al.* and Bransteitter with any reasonable expectation of success.

In a 2006 decision, the Federal Circuit further clarified that the motivation to combine can arise even though there is nothing in the references themselves that suggests that they should be combined. For example, implicit motivation to combine may come from: i) the knowledge generally available to one of ordinary skill in the art; ii) the common general knowledge and established scientific principles; and iii) the universal desire to enhance commercial properties. *Dystar Textilfarben GMBH & Co. Deutschland KG v. C.H. Patrick, Co.*, No. 06-1088 (Fed. Cir. October, 2006).

Applicants respectfully submit that to determine the knowledge generally available to one of ordinary skill of the art, one must establish who is "one of ordinary skill" and what is the pertinent art.

With respect to one of ordinary skill in the art, the law is clear. "*The hypothetical 'person having ordinary skill in the art' to which the claimed subject matter pertains would, of necessity have the capability of understanding the scientific and engineering principles applicable to the pertinent art.*" Ex parte Hiyamizu, 10 USPQ2d, 1393, 1394 (Bd. Pat. app & Inter. 1988). "*The Examiner must ascertain what would have been obvious to one of ordinary skill in the art at the time the invention was made, and not to the inventor, a judge, a layman, those skilled in remote arts, or to geniuses in the art at hand.*" *Environmental Designs, Ltd. v. Union Oil Co.,* 713 F.2d 693, 218 USPQ 865 (Fed. Cir. 1983), *cert. denied*, 464 U.S. 1043 (1984).

Applicants submit that in the present case, the problem to be solved by the present invention was to develop new DNA testing methods for determining the methylation status in target genomic or mitochondrial DNA. Therefore, in the present case, one of ordinary skill in the art is a person performing DNA testing. The Examiner alleges that the person of ordinary skilled in the art would have be motivated to modify the method of Gitan *et al.* to incorporate the enzyme of Bransteitter *et al.* At page 8 of the Office Action, the Examiner states that Bransteitter *et al.* teach a method wherein the AID enzyme differentially modulates the activity of single stranded DNA comprising cytosine and methylated cytosine. Applicants respectfully disagree with this statement. The article by Bransteitter *et al.* is directed to the study of the AID enzyme and its role in B cells. Specifically, the authors investigated the effect of the enzyme on somatic hypermutation i.e. a high frequency of mutation that occurs in the gene segments encoding the variable regions of antibodies during the differentiation of B lymphocytes into antibody producing plasma cells. In fact, the specific experiments cited by the Examiner involved the determination that an inhibitory RNA is bound to the AID enzyme and that this inhibitory RNA prevents the AID enzyme from acting on single stranded DNA. The article does not describe, as alleged by the Examiner, a method for determining the presence or level of alkylated cytosine in DNA.

Applicants submit that a person performing DNA testing, when searching the prior art, would have been unlikely to identify the article by Bransteitter *et al.* which is directed to a different field of endeavor, namely B cell immunology. Accordingly, Applicants respectfully submit that the Bransteitter *et al.* reference is not, "reasonably pertinent to the particular problem" as a matter of law and would not have logically commended itself to the inventor's attention when considering his problem.

Applicants submit that in order for the Examiner to rely on a reference under 35 U.S.C. § 103, it must be analogous prior art. The Examiner must determine what is "analogous prior art" for the purpose of analyzing the obviousness of the subject matter at issue. "In order to rely on a reference as a basis for a rejection of an applicant's invention, the reference must either be in the field of applicant's endeavor, or if not, then reasonably pertinent to the particular problem with which the inventor was concerned" See, e.g. *In re Oetiker*, 977 F.2d 1443, 1446, 24 USPQ2d, 1443, 1445 (Fed. Cir. 1992); *In re Deminski*, 796 F2.d 436, 230 USPQ 313 (Fed. Cir. 1986); *In re Clay*, 966 F.2d 656, 659, 23 USPQ 2d 1058, 1060-61 (Fed. Cir. 1992. ("A reference is reasonably pertinent if, even though it may be in a different field from that of the inventor's endeavor, it is one which, because of the matter with which it deals, logically would have commended itself to an inventor's attention in considering his problem.") (*Wang Laboratories Inc. v. Toshiba Corp.,* 993 F.2d 858, 26 USPQ2d 1767 (Fed. Cir. 1993).

Applicants respectfully submit that under the current controlling law, the reference to Bransteitter *et al.* should be considered nonanalogous art. Applicants submit that the Bransteitter *et al.* is a study in fundamental B cell biology. It is reasonable to assume that this type of study would not form part of the prior art base nor form part of the common general knowledge of a scientist performing DNA testing.

Additionally, with respect to the common general knowledge and established scientific principles, the law provides that "[t]he rationale to modify or combine the prior art does not have to be expressly stated in the prior art; it may be reasoned from knowledge generally available to one of ordinary skill in the art, established scientific principles, or legal precedent established by case law. *in re Fine,* 837 F.2d 1071, 5 USPQ2d 1596(Fed. Cir. 1988); *In re Jones*, 958 F.2d 347,

21 USPQ2d 1941 (Fed. Cir. 1992), *In re Eli Lily & Co.,* 902 F.2d 943, 14 USPQ2d 1741 (Fed. Cir. 1990); *In re Nilssen*, 851 F.2d 1401, 1403, 7 USPQ2d 1500, 1502 (Fed. Cir. 1988). The rationale supporting an obviousness rejection may be based on common general knowledge in the art or "well known" prior art. The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well known" in the art. *In re Ahlert*, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970).

Applicants submit that, even assuming *arguendo*, that the Examiner's reasoning has certain logic and scientific basis, that basis alone cannot constitute a ground of rejection of obviousness under the law. "Although the theoretical mechanism of an invention may be explained by logic and sound scientific reasoning, this fact does not support an obviousness determination unless logic and scientific reasoning would have led one of ordinary skill in the art to make the claims invention." *Ex parte Levengood*, 28 USPQ2d 1300 (Bd. pat. App. & Inter. 1993).

Applicants respectfully submit that in the present case, one of ordinary skill in the art (i.e. a person performing DNA testing) would not have been led to combine the prior art to make the claimed inventions as a result of established scientific principles or common general knowledge. Established scientific principles and common general knowledge of a person performing DNA testing would not include fundamental B cell immunology as taught in Bransteitter *et al.* and would not provide any rationale or motivation to combine the reference with the teaching of Gitan *et al.*

In view of the above arguments, Applicants respectfully submit that the Examiner has unreasonably combined the references and it was not obvious to one of ordinary skill in the art at the time the present application was filed to use an enzyme in a method for detecting the presence or level of alkylated cytosine in a sample of genomic or mitochondrial DNA.

The Examiner also rejects Claims 1 and 4-13 are rejected under 35 U.S.C. § 103 as allegedly unpatentable over Gitan *et al.* (*Genome Research*, 2001, 12, 158-164) in view of Bransteitter *et al.* (*PNAS*, 2003, 100, 4102-4107) further in view of Kuhn *et al.* (*J. Am. Chem. Soc.*, 2002, 124, 1097-1103).

The combination of Gitan *et al.* and Bransteitter *et al.* is addressed above. Kuhn *et al.* provide methods for separating double stranded DNA into two single strands. Kuhn *et al.* do not, however, provide any teaching or information regarding differentially modifying alkylated cytosine and cytosine present in single stranded DNA. Accordingly, it is respectfully submitted that the addition of Kuhn *et al.* do not cure the deficiencies of the combination of Gitan *et al.* and Bransteitter *et al.* Therefore, it is respectfully submitted that the claims are inventive over the combination of Gitan *et al.* in view of Bransteitter *et al.* further in view of Kuhn *et al.*

The Examiner has also rejected Claims 1 and 32 for allegedly being obvious in light of Gitan *et al.* (*Genome Research*, 2001, 12, 158-164) in view of Bransteitter *et al.* (*PNAS*, 2003, 100, 4102-4107) further in view of Opdecamp *et al.* (*Nucleic Acids Research*, 1992, 20, 171-178).

The combination of Gitan *et al.* and Bransteitter *et al.* is addressed above. Opdecamp *et al.* teach the identification of methylated DNA using methylation-sensitive restriction enzymes. In the claimed method the enzyme is acting on single stranded DNA. It is well understood that restriction enzymes only recognize and cleave double stranded DNA. Accordingly, none of the enzymes referred to in Opdecamp *et al.* differentially modify cytosine and alkylated cytosine in single stranded DNA. Accordingly, it is respectfully submitted that the present claims are inventive in view of the combination of Gitan *et al.* in view of Bransteitter *et al.* further in view of Opdecamp *et al.*

The Examiner has also rejected Claims 1 and 34 under 35 U.S.C. § 103 as allegedly being unpatentable over Gitan *et al.* (Genome Research, 2001, 12, 158-164) in view of Bransteitter *et al.* (PNAS, 2003, 100, 4102-4107) further in view of Paulson *et al.* (J. Virol., 1999, 73, 9959-9968).

The combination of Gitan *et al.* and Bransteitter *et al.* is addressed above. Paulson *et al.* use bisulfite modified DNA which is then amplified using PCR to detect sites of methylation in the viral genome. Accordingly, the methodology of Paulson *et al.* is similar to that of Gitan *et al.* and which fails for the same reasons. As with Gitan *et al.* there is no disclosure in Paulson *et al.* for the use of an enzyme which differentially modifies cytosine and alkylated cytosine. Further,

the use of an enzyme in the methods described by Paulson *et al.* would also render their methods as unsatisfactory for the intended purpose i.e. the method would fail to work.

Therefore, in view of the foregoing remarks, withdrawal of the rejections raised under 35 U.S.C. § 103 are respectfully requested.

CONCLUSION

In view of the foregoing, the application is respectfully submitted to be in condition for allowance, and prompt favorable action thereon is earnestly solicited.

If there are any questions regarding this amendment or the application in general, a telephone call to the undersigned would be appreciated since this should expedite the prosecution of the application for all concerned.

The fee in the amount of $1,920.00 is being paid simultaneously herewith via EFS representing payment of a three-month extension of time pursuant to 37 C.F.R. §1.17(a)(3) ($1,110.00) and payment of a Request for Continued Examination pursuant to 37 C.F.R. §1.17(e) ($810.00). Please charge any deficiency in fees or credit any overpayments to Deposit Account No. 50-3211 (Docket No. 22238.0004).

Respectfully submitted,

Date: September 17, 2009

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